

Additional questions for Eisenmann lectures
Updated 082312

1. I was wondering what the differences in function were for Tropomodulin and Phalloidin in regards to arresting actin depolymerization

Tropomodulin is a protein that can bind to actin as well as tropomyosin. Tropomodulins are a family of actin capping proteins that block actin association and dissociation at the slow growing (pointed or -) ends of actin filaments. In the presence of tropomyosin it acts as a capping protein to the – or pointed end to inhibit addition of actin to the ends as well as depolymerization. Tropomyosin protects actin filaments from severing by ADF/cofilin and stabilizes sarcomeric actin organization (recall that Actin depolymerizing factor (ADF)/cofilin enhances disassembly of actin filaments).

Phalloidin is a neurotoxin from *A phalloides* mushroom. It works differently from tropomodulin in that it stabilizes F-actin filaments by inhibiting actin monomer dissociation at both + and – ends. This happens because phalloidin binds to the F-actin polymer much more strongly than monomers, negatively impacting the dissociation of monomers from the filament ends- so no depolymerization. It's also thought that phalloidin binding to F-actin causes a conformational change of sorts at the polymer ends that inhibits the addition of new monomers.

2. In regards to your lecture on the cytoskeleton, there was a lot of information covered, and I am having problems determining what is important. How much detail should we go into when studying? Can you give me any advice on how to review the material?

For example, when you talked about intermediate filaments, you mentioned the major classes. Should we be able to separate them into Type I-V? Also, in the learning objectives, #6 just says "list tubulin binding drugs". Does that mean we don't have to worry about how the drugs function?

The general rule of thumb for the Block Courses is that if it's the lecture slides, it is fair game for an exam question. I draw a lot of my lecture material from the Step 1 review books from the last 2-3 years, and have seen questions of the individual IF classes and their respective proteins on the practice tests for Step 1. So my advice would be to have an understanding of it, and know that there are 5 classes, separated by function and where the big players fall into this picture (for my lectures this was keratin and lamins- but I cannot speak for lectures still to come from other professors). Am I asking folks to draw the structure of an IF and label its parts- no. Just to understand a little bit about its structure is sufficient (polar, head to tail configuration, 10um diameter). But please be familiar with what they look like by EM (relative sizes, locations) as I see these in practice tests...

re: tubulin binding drugs, these drugs are also featured in step 1 exams, and I have seen the in at least 3 separate review guides. I would know briefly how they are working so you can answer a question like the vignette from pg 114.

I know it's a lot of info to process, but this is the nature of the game right now. I would HIGHLY recommend that you have a look at the USMLE Step 1 exams (practice exams/Qs are available through the UT library Med Student Yr 1/2 portal) and grab a study guide so you can see the type of questions that are being asked and the depth of the info that needs to be recalled. I really like the 'First Aid for the USMLE Step 1, 2011 guide' that I borrowed from my MD/PhD student that passed her exams last summer. The data are presented as pretty succinct snippets, which is how you need to roll now. There are lots of them available for check out through the UT library. I would also recommend that you have a chat with a M2 to see if there is advice they could lend you, as they were in your shoes last year.

3. Pg 30 (last slide of lecture 1) says:

- **Cell death is characterized by typical nuclear changes.**
- **Cell membrane rupture is another sign.**

From the earlier sides, it seems *apoptosis only *has nuclear changes and *necrosis only* has cell membrane rupture.

Am I correct in thinking that "cell death" is inclusive of apoptosis and necrosis?

This needs to be clarified on the slides for next year since as presented it is an incomplete statement. The rupture is distinct to necrosis, where cell injury can basically cause cell bursting in some instances. This rupture drives an inflammatory response. Apoptosis is a more controlled release of cell membranes in the form of apoptotic bodies, which exclude the cell contents from the neighboring cells. No inflammation for apoptosis.

4. In your lecture "Cell Structure and Function" you covered a slide (p. 91 of handout) titled "Assembly and Structure of Actin Filaments *in vitro*". I wanted to ask you about the motility of the cell with regards to the nucleation site. In the "treadmill motion model" we discussed that monomers from the (-) end are phosphorylated to add to the (+). How does the nucleation site remain part of the f-actin structure if monomers are lost at the (-) end and added to the (+) end? It seems to me that at a certain point, the nucleus would be "treadmilled" off the molecule. Thank you for your time.

Good question. Basically you can think of the F-actin nucleus in terms of a few monomers tied together that can "seed" new actin polymerization if you were

starting from scratch in vitro in a test tube. In order to get the filament to polymerize, the most efficient thing to do is to add these pre-formed seeds to the reaction to accelerate the reaction, since self assembly of G-actin monomers into polymers is a very inefficient process.

So in the treadmilling model in vitro, yes, essentially the nucleus would fall off at some point. We can see this by creating F-actin seeds that have incorporated fluorescently labelled G-actin monomers, and then one can trace the movement of the nuclei relative to the growing filament by using live cell fluorescent TIRF microscopy (very cool stuff). However, even if the nuclei "falls off" during treadmilling, there is a polymerized polar F-actin filament that still acts as a nucleus or seed to accelerate the addition of new monomers.

In vivo in cells, the seeds, nuclei or "free barbed ends" as they are called, can be created from severing pre-existing filaments. That is, sever a giant filament and now you have two fresh ends that act as nuclei that are site for new monomer addition.